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Chapter 7

Primary and Metastatic Tumours of the Liver: Expanding Scope of Morphological and Immunohistochemical Details in the Biopsy

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Additional information is available at the end of the chapter

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1. Introduction

Evaluation of liver biopsy for tumour diagnostics is a highly practical task with major clinical influence. The liver is frequently affected by wide spectrum of neoplasms including benign tumours as well as primary malignancies [1-3]. In addition, due to the rich dual blood flow to liver, secondary malignant tumours also often develop here. In order to ensure the optimal management of the patient, a correct diagnosis is necessary. At present, biopsy is the gold standard in oncology [4-5].

The scope of liver neoplasms can be following. The benign tumours include hepatic adenoma, bile duct adenoma, cavernous haemangioma and angiomyolipoma, among others. The primary liver malignancies embrace hepatocellular carcinoma [6,7], cholangiocarcinoma [3] and hepatoblastoma [8]. The diagnostics of hepatocellular carcinoma (HCC) is especially urgent topic due to high incidence in Asia and rising – in Europe and USA, possibly because of high prevalence of chronic hepatitis C [4,9]. Also, prognostic data should be reported including the features of early vs. progressed HCC, presence of stem cell immunophenotype, multicentric growth or metastatic spread [7]. Among mesenchymal malignant tumours, epithelioid haemangioendothelioma and angiosarcoma [10,11] are notable. Metastatic tumours represent the bulk of malignancies in Western countries [2]. Cystic liver tumours include biliary cystadenoma and biliary cystadenocarcinoma [12-14].

Most of the above mentioned neoplastic processes can be diagnosed in core biopsy. The key aspects include the following. First, the biopsy must be representative regarding the biologi-

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cal process and radiologically detected changes [15]. Further, the obtained tissue must be subjected to adequate technological process. Innovations here allow shortening the turnover time significantly. Next, the evaluation of morphology must be done searching for the characteristic traits of the above noticed tumours. However, due to the limited tissue amount in the biopsy, the tumour architecture sometimes is difficult to identify embarrassing the distinction between nodular hyperplastic process, benign tumour or low-grade malignancy. In contrast, high-grade malignancies can show significant cytological atypia by few signs of differentiation embarrassing the detection of histogenesis [6] and the distinction between primary and metastatic tumour.

Immunohistochemical markers as glypican-3 [1], Hep Par 1 [3,6], CD10 [3], alpha-fetoprotein [6] and TTF-1 [16] are useful in the HCC diagnostics. Alterations of CD31 and CD34-positive endothelial cell network reflect vascular remodelling during hepatic carcinogenesis [7]. Cytokeratin (CK) 19 and 7 are characteristic for cholangiocellular carcinoma [3]. In metastases, organospecific markers including CDX2, mammaglobin, nuclear expression of TTF-1 or presence of neuroendocrine markers can confirm extra-hepatic origin [17]. As colorectal, breast, lung and neuroendocrine cancers are frequent cause of metastatic liver damage [2] high diagnostic value of immunohistochemistry (IHC) can be expected. However, the exact detection of histogenesis can be difficult with metastatic pancreatic or gastric tumours and high-grade malignancies. IHC is mandatory for the diagnostics of haematological neoplasms and epithelioid haemangioendothelioma. Assessment of tumour biological potential can be done by IHC, evaluating Ki-67, Cyclin D1, FOXJ1, stem cell markers, matrix metalloproteinases and other markers [7-8,18-22]. Novel markers appear continuously as heat-shock protein 70 [23].

Nowadays, pathology is not any more purely descriptive but it is becoming more functional and clinically relevant. The classic morphologic characteristics must be combined with integrated evaluation of neoplastic process in the liver, including histogenesis, grading, clonal changes, type and extent of vascularisation, immunophenotype, heterogeneity, prediction of treatment sensitivity and the clinical behaviour [7]. New technologies as proteomic profiling and genomic marker analysis should be applied in the evaluation of liver tumours [4]. MicroRNA studies can lead to new findings in cancer pathogenesis and prediction of treatment efficacy [24,25].

The aim of the following chapter is to describe morphological and immunohistochemical characteristics of primary and secondary liver tumours in order to develop logistic basis for differential diagnosis of these processes in biopsy materials. Short discussion about the genesis and clinical course of each tumour will be included as well.

2. Benign epithelial liver tumours

Liver cell adenoma and bile duct adenoma will be discussed here. The regenerative processes with the emphasis on focal nodular hyperplasia are described considering the differential diagnosis.

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2.1. Liver cell adenoma and its differential diagnosis with focal nodular hyperplasia

Liver cell adenoma or hepatic adenoma is defined as benign tumour arising from hepatocytes. The epidemiology is characterised by female predominance (90%) and strong association with oral contraceptive use [26-27] as 85% of affected persons have such history. Liver cell adenoma was rare before the era of oral contraceptives [27]. At present, the incidence has increased but is still low: 3-4 /100 000 per year in long-term users of oral contraception [27-29]. The patients mostly are 20-39 years old. The other risk factors of hepatic adenoma include androgen burden. The tumours can also arise spontaneously or occasionally can be related to glycogen storage diseases or diabetes mellitus. Clinically, the patients mostly are symptomatic. Abdominal fullness can be attributed to the presence of mass lesion; pain can be caused by necrosis [27]. Rupture and bleeding (40%) represent dangerous complications [27,29-31]; the risk of these events is increased in pregnant ladies affected by liver cell adenoma due to prior use of hormonal contraceptives. Risk of malignant transformation also is recognised [29,32]. By literature analysis, Farges and Dokmak concluded that 5% of resected hepatic adenomas bear HCC foci [32]. The risk of malignant transformation is higher in adenomas exceeding the size of 5 cm irrespectively of the number of adenomas as well as in males. Grossly, liver cell adenomas are mostly unifocal (80%) and subcapsular. The tumours can be quite large (5-20 cm). In most cases (75%) adenomas are encapsulated [27]. However, the capsule can be thin or absent [10]. In contrast to HCC, adenomas usually are not associated with cirrhosis [31]. Otherwise, radiological similarities exist between adenoma and HCC as both can be large, have rich vascularity and can undergo necrosis [31]. Microscopically, the tumour is composed by hepatocytes lacking anaplasia and arranged in thin (1-2 cells) trabeculae [27,29]. Cellular atypia and macrotrabeculae must be absent. Single arterioles, a pair of arteriole and venule or isolated biliary ducts are scattered throughout the lesion. However, well-formed triads enveloped in connective tissue are absent within the lesion. The tumour can be distinguished from normal liver by larger size of neoplastic cells, presence of capsule and lack of triad-containing portal tracts. Steatosis, hydropic degeneration or Mallory hyaline can be observed. Fibrous tissue, haemosiderin and calcifications can develop in the consequence of haemorrhage. The immunophenotype is characterised by expression of Hep Par 1 and other antigens that confirms the hepatic origin and by lower proliferation than in HCC. Molecular typing is emerging for liver cell adenoma as well. At present, up to 4 molecular types are identified:

1. hepatic adenoma with *TCF1* gene inactivation;
2. inflammatory hepatic adenoma;
3. beta-catenin-mutated non-inflammatory hepatic adenoma;
4. hepatic adenoma not displaying any before described feature or unsuitable for analysis [29].

The hepatic adenomas with *TCF1* gene mutation comprise 35-40% of liver cell adenomas. The patients are female. The tumour loses the expression and functions of hepatocyte nuclear factor 1 (HNF1) encoded by *TCF1* gene. Inactivation of the gene can be caused by mutation in both alleles or by combination of a mutation and 12q deletion leading to loss of heterozygosity in the corresponding region [33]. Germ-line mutation of *HNF1* gene man-

ifests as maturity-onset diabetes of the young (MODY), type 3, in association with liver adenomatosis [34]. However, the spectrum of *HNF1A* somatic mutations in liver cell adenoma differs from that in patients with MODY3 and suggests genotoxic damage [35]. By IHC, loss of liver fatty acid binding protein can be observed. Not surprisingly, the adenomas show steatosis [29].

Inflammatory hepatic adenomas constitute 50% of liver cell adenomas and can be associated with obesity, smoking and alcohol use. Pathogenetically, inflammatory hepatic adenoma is characterised by IL-6 pathway activation centred on gp130 protein in IL-6 receptor. The receptor can be subjected to ligand-independent activation due to mutation in *IL6ST* gene, or the levels of gp130 can be elevated. The IL-6 receptor activation leads to recruitment of inflammatory cells through gp130-mediated production of chemokine CCL20. The mutation was found in 60% of inflammatory adenomas [36]. However, the IL-6 pathway activation is universal in the inflammatory hepatic adenoma. Microscopically, inflammatory infiltrates are observed in addition to the architecture and cytologic details of adenoma. Occasional bile ductules, dilated sinusoids and arterioles can be present. Haemorrhage is frequent. By IHC, expression of acute phase reactants serum amyloid A and C-reactive protein is marked [29].

A group of hepatic adenomas is associated with *beta-catenin* mutation [37-38].The beta-catenin pathway is not affected in *TCF1* inactivated group [29,38]. Beta-catenin activation can be assayed by immunohistochemical over-expression of glutamine synthetase or by aberrant nuclear localisation of beta-catenin. However, the tumours can show dysplastic changes more characteristic for HCC thus possibly this group will be reclassified into well-differentiated HCC [29,36].

The last group of hepatic adenomas (5%) lacking *TCF1* inactivation, inflammatory signature and beta-catenin mutation [29] could represent distinct group with peculiar pathway of molecular pathogenesis or result of technological shortcomings.

The differential diagnosis of hepatic adenoma in biopsy includes low-grade HCC and hyperplastic lesions like focal nodular hyperplasia, nodular regenerative hyperplasia and partial nodular transformation [27].

Focal nodular hyperplasia (FNH) is a comparatively frequent differential diagnosis of hepatic adenoma. The FNH incidence is estimated as 3% [29-30,39]. FNH is characterised by presence of hypervascular stellate scar in liver parenchymal nodule. The blood vessels are located in the middle of star-like fibrous tissue while the periphery is occupied by proliferating bile ductules. The morphologically remarkable abundant vascularity is in accordance with the hypothesis of the FNH origin due to microscopic arterial malformation [40-42]. The crucial difference between FNH and adenoma is pathogenetic as the former is thought to be hyperplastic lesion, while adenoma is a neoplasm. The presence of stellate scar and lack of peripheral capsule in FNH contrasts with presence of peripheral capsule and almost complete lack of connective tissue or portal triads within adenoma. If the architecture is incompletely represented in the biopsy, molecular characteristics should be able to discriminate between the two inherently different processes, the hyperplasia and tumour. The immunohistochemical markers of biliary differentiation have been employed in the differential diag-

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nostics between FNH and hepatic adenoma. As described by Walther and Jain, CK19 and CD56 detect rich network of proliferating biliary ducts in the fibrous septa of FNH but reveal only few isolated ducts within the parenchyma of hepatic adenoma. Expression of CK7 is remarkable for the focal presence in parenchyma of liver cell adenoma in contrast to FNH while both lesions show expression of CK7 in biliary ducts. Thus, panel of CK19, CD56 and CK7 can be advised to solve the differential diagnosis in core biopsy [29]. Immunohistochemical expression pattern of glutamine synthetase differs between normal liver tissue, FNH and liver cell tumours as well. In healthy tissue, glutamine synthetase is present in perivenular hepatocytes. These positive areas are expanded in FNH [39]. In hepatic adenomas, glutamine synthetase expression is either diffuse of negative. In the last situation, the negativity in the tumour can be incomplete, with focally preserved expression in the tumour periphery [29] and thus difficult to interpret, especially in small biopsies where the preserved positive focus seems to be dominant.

In nodular regenerative hyperplasia, the liver contains many small regenerative nodules. Partial nodular transformation affects hilar area and is characterised by group of regenerative nodules surrounded by fibrous tissue [27].

Considering the differential diagnosis with HCC, thick trabecular cords, cytologic anaplasia and invasive growth reveal the malignant biological potential. The thickening of trabeculae is defined as presence of more than 2 cell layers in the trabeculae. The anaplasia is recognised by nuclear hyperchromasia, prominent nucleoli and increase in the nucleo: cytoplasmic ratio. Presence of mitoses practically excludes the diagnosis of hepatic adenoma. Atypical mitoses are absolute evidence of malignancy. The invasive growth can manifest as invasion through the capsule, infiltration into liver parenchyma and true invasion into blood vessels [27].

2.2. Bile duct adenoma

Bile duct adenoma is defined as a benign neoplasm of portal bile ducts. The epidemiologic data suggest rare occurrence. However, as the tumours mostly are small and asymptomatic [27], the true incidence and prevalence is unknown. Grossly, bile duct adenomas are mostly solitary (83%), subcapsular (95%) and small (below 1 cm). By light microscopy, the lesion is characterised by demarcated proliferation of bile ducts lacking atypia. The immunophenotype repeats the staining characteristics of biliary ducts exhibiting expression of cytokeratins 7 and 19 [27]. The differential diagnosis can include small foci of low-grade cholangiocarcinoma or metastatic low-grade adenocarcinoma, but the benign cytological appearance is helpful. Von Meyenburg hamartoma differs from bile duct adenoma, as the hamartomas would be multiple and show traits of cholestasis. However, the exact separation might not be of crucial importance due to benign course of biliary adenoma and pathogenetic suggestion that biliary adenoma represent a reactive process rather than true neoplasm.

3. Malignant epithelial primary liver tumours

Three primary liver tumours are of utmost importance. Hepatocellular carcinoma is the most frequent primary epithelial liver tumour with grave prognosis. Cholangiocarcinoma ranks second by the incidence except for endemic regions. Hepatoblastoma is notable for the occurrence in the infancy.

3.1. Hepatocellular carcinoma

Hepatocellular carcinoma is defined as malignant tumour developing from hepatocytes and/or showing hepatocellular differentiation. It is the most common primary malignant tumour of the liver constituting 80-85% of primary epithelial liver malignancies [29,43]. Considering the epidemiology, the worldwide burden of hepatocellular carcinoma can reach 1 million of new cases per year. The incidence shows major geographic differences. HCC is the 2nd most common cancer in Asia, and the 4th – in Africa [10]. The annual age-standardised incidence is the highest in East Asia, including China and Japan. Low-risk areas comprise Europe, esp. northern and western parts; North and South America, Australia and New Zealand [10].The age-adjusted incidence rates in Mozambique are as high as 112.9 and 30.8/100 000 in males and females, respectively. In China these values reach 34.4 and 11.6. In contrast, the age-adjusted incidence rates in British males and females are 1.6 and 0.8, respectively [31]. The HCC risk factors include liver cirrhosis independently of cause, chronic hepatitis B or C, ethanol consumption and non-alcoholic liver steatosis as well as mycotoxins. The aflatoxin B1 or other mycotoxins produced by *Aspergillus* fungi could be responsible for part of HCC in areas where grains, rice and peanuts are stored in hot and humid conditions [31]. Most of HCC cases develop from dysplastic cirrhotic nodule [29], thus the differential diagnostics between dysplastic nodule and cancer represent evaluation of one point in a complex road of pathogenesis. Clinically, most of the patients approach doctor due to symptoms attributable to mass lesion in the liver (abdominal pain, sensation of fullness), tumour-related intoxication (weight loss, weakness, lack of appetite) and loss of liver functions (jaundice). Alternatively, the symptoms can be related to pre-existing cirrhosis and the tumour could be identified during routine control of cirrhotic patient or during workup for unspecific or unrelated symptoms [31]. Radiologically, the number and size of tumour masses can be evaluated. Ultrasonography can be used for screening. Typical findings regarding vascularity include hypervascularity and thrombosis of portal vein, frequently due to invasion. If it is necessary to confirm invasion into portal vein, biopsy can be obtained from it [31].

By microscopy, the typical patterns include trabecular, acinar and ductular structure. The neoplastic cells in low-grade cases resemble liver cells by possessing wide eosinophilic cytoplasm and distinct cell borders. Nuclear atypia is present and nucleo: cytoplasmic ratio is increased, although to different degree. Mitoses can be present; atypical mitoses can be observed (Figure 1). The architecture shows unequivocal deviations from normal structure such as thick trabeculae with more than 2 cell layers (in contrast to adenoma), solid areas, duct-like or gland-like structures. However, careful evaluation of the architecture under

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high power magnification must be carried out. There are many secondary phenomena raising the similarity between HCC and liver tissue: presence of macrovesicular or microvesicular fat, Mallory hyaline and bile. The capillaries can be dilated [27]. Among the histochemical staining methods, absent reticulin staining [44] is characteristic. PAS stain can reveal glycogen and intracytoplasmic globules; the latter structure remains positive after diastase digestion [27,44]. With some experience, morphology is helpful to distinguish finely granular glycogen or rounded globules in HCC from mucus droplets in metastatic adenocarcinoma or cholangiocarcinoma.

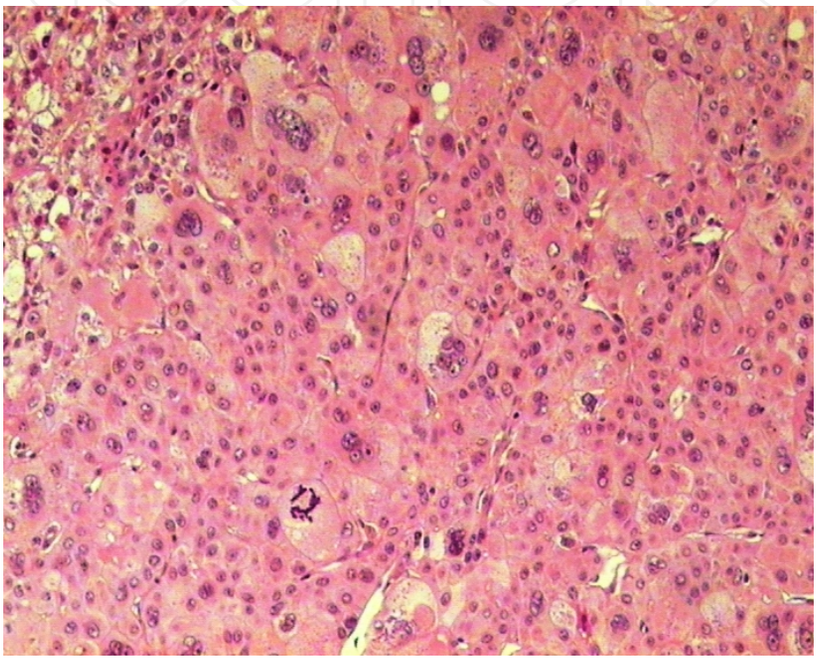


Figure 1. Hepatocellular carcinoma displaying marked cytologic atypia. Note the presence of atypical mitosis. Haematoxylin-eosin (HE), original magnification (OM) 100x.

Fibrolamellar hepatocellular carcinoma (FLHC) has distinctive aetiology, epidemiology and course. The general HCC risk factors are not associated with this subtype [31]. FLHC is rare, constituting only 1-4% of HCC [27]. It is less common in high-risk areas than in North America and Europe. The patients are young adults or even children [10]. FLHC is diagnosed at the mean age of 25 years in contrast to mean age of 52 years in typical HCC patient group [27]. Controversial data are reported about the sex predilection: some but not all authors have noted that females are mostly affected [10,31]. Clinically, symptoms attributable to liver enlargement, parenchymal damage (elevated liver enzyme level) or tumour-related intoxication (weight loss or fever) can be present. Cirrhosis is absent. The tumour can be multifocal, and metastases can affect lungs and regional lymph nodes [31]. The histological structure is remarkable for the lamellar fibrosis. The stroma is composed of thick, parallel strands of hyalinised collagen [27]. The cells are large, polygonal, with wide eosinophilic cytoplasm. The vesicular nuclei possess large nucleoli. Cytoplasmic pale bodies are more frequent (up to 50% of cases) and more abundant than in other types [10,27]. The pale bodies

are rounded and very lightly eosinophilic thus staining paler than the surrounding cytoplasm. These structures represent cystically dilated endoplasmic reticulum. Pale bodies can be positive for fibrinogen by IHC. The immunophenotype is remarkable for diffuse expression of CK7. The hepatocellular differentiation can be confirmed by Hep Par 1; alpha-fetoprotein is present in approximately 20% of cases. The FLHC prognosis is better than in the general group. The mean survival is 32 months in contrast to 5.9 months in trabecular HCC [27]. However, it is found that the beneficial prognosis of FLHC is different from cancer in cirrhotic liver but not from HCC in the absence of liver cirrhosis [10].

IHC has an important role in the diagnostics of HCC. Frequently tested antigens include glypican-3, Hep Par 1, alpha-fetoprotein, CD10, carcinoembryonic antigen CEA, TTF-1, arginase-1, evaluation of cytokeratins and endothelial network as well as MOC-31 and markers of extra-hepatic tumours.

Glypican-3 is a cell surface protein [1] that is involved in the control of cell proliferation and survival. Glypican-3-knockout mice exhibit alterations in Wnt signalling [45]. Glypican-3 also interacts with Hedgehog signalling pathway [46]. In the practical surgical pathology, the value of glypican-3 is associated with the cancer diagnostics as it is expressed in 70-75% of HCC but not in benign liver tissue [48-49] or cholangiocellular carcinoma [1]. Hepatoblastoma can be positive as well. However, glypican-3 can be expressed in metastatic melanoma [50], ovarian clear-cell carcinoma [51], choriocarcinoma, yolk sac tumour [52-53] as well as in blastomas including neuroblastoma and Wilms' tumour [54]. In addition, 10% of gastric cancer cases are positive for glypican-3 [55]. In melanoma, 80% of tumours contain detectable level of glypican-3 protein and mRNA [1]. Regarding ovarian cancer, the rate of glypican expression could be as high as 18% of all ovarian cancer cases and 60% of clear cell carcinoma cases [51]. However, negative reports regarding clear cell carcinoma of ovary are published as well [53]. Glypican-3 is silenced in breast cancer, lung adenocarcinoma and mesothelioma [56-58]. Another problem has been highlighted by Abdul-Al *et al*., who have described frequent granular cytoplasmic expression of glypican-3 in chronic active hepatitis C [59]. Regenerative changes were suggested as the explanation. Authors emphasized that membranous staining was not observed in hepatitis [59]. Glypican-3 has prognostic significance in HCC as it is associated with poor prognosis [60] and shorter recurrence-free period after liver transplantation [49]. The applications of glypican-3 could extend beyond liver biopsy – and return to it. It could possible to use glypican-3 plasma levels for diagnostics and monitoring of HCC [61-63]. Immunotherapy could be guided towards glypican-3; the present research is exploring both antibody and cell-based immunological mechanisms [64-65]. Cancer vaccine could be generated against this molecule [1]. Glypican-3 is among genes that are distinctly expressed in liver cancer stem cells; it is suggested that glypican could be promising candidate for gene therapy without inducing damage to normal liver stem cells [66].

Hep Par 1 is positive in normal liver, liver adenomas and HCC. The antibody was developed in 1993 using an immunogen from failed liver allograft. The target antigen has been identified as carbamoyl phosphate synthetase. This enzyme catalyses the rate-limiting step in the urea cycle and is located in the mitochondria [67]. The specifity and sensitivity of this

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marker in HCC diagnostics exceeds 80% and has reached 90% in several studies [6,67]. Unfortunately, sensitivity is lower in high-grade HCC. The expression in non-hepatocellular tumours including colorectal, pancreatic, breast, urothelial, prostate cancer, neuroendocrine tumours, renal cell carcinoma, melanoma and angiomyolipoma is either negative or focal. However, few gastric, colorectal and lung adenocarcinomas can be positive [6,67]. In the biopsy material, heterogeneity in the HCC can cause diagnostic problems [6].

Arginase-1 is an enzyme involved in the urea cycle as well. It is found in benign hepatocytes and hepatocellular neoplasms. The antibody has received high sensitivity estimates of 96% and favourable performance characteristics [68,69].

Alpha-fetoprotein is an oncofetal protein produced by the liver and yolk sac endoderm. The antigen is remarkable for expression in malignant hepatocellular tumours (Figure 2) in contrast to benign liver tissue, and for the high specifity. However, sensitivity is low (30-50%) and heterogeneity adds further problems in biopsy evaluation [6]. Nevertheless, positive expression is valuable.

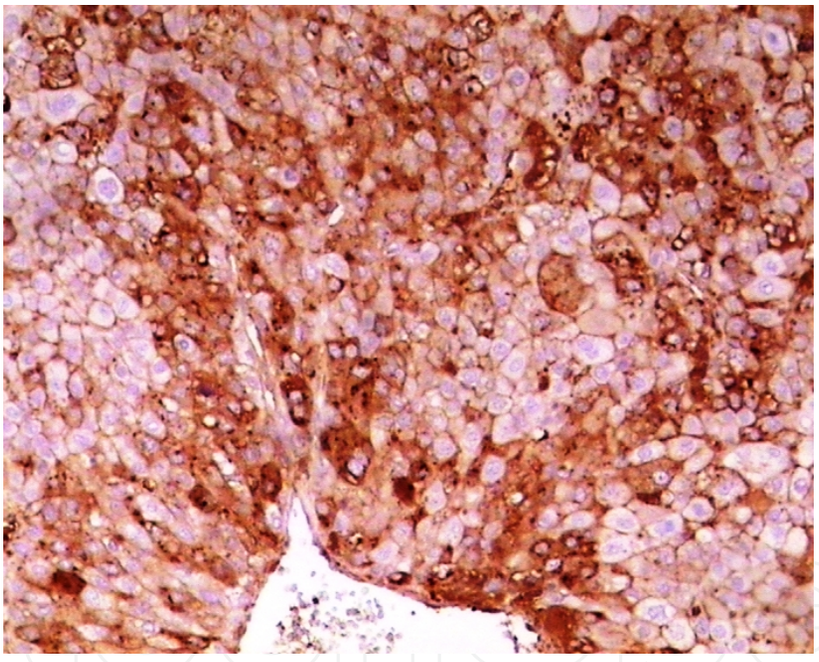


Figure 2. Heterogeneous intense cytoplasmic expression of alpha-fetoprotein in hepatocellular carcinoma. Immunoperoxidase (IP), anti-alpha-fetoprotein, OM 100x.

Polyclonal antibodies against carcinoembryonic antigen (CEA) yield positive reaction more than in 70% of HCC cases, while monoclonal anti-CEA only rarely stains HCC. Reactivity with polyclonal CEA antibodies mostly is observed in canaliculi; this pattern can be observed in benign or malignant liver tissues and is attributable to cross-reaction with biliary glycoprotein on the canalicular surface [67]. The canalicular pattern is specific for HCC and can be used to exclude cholangiocarcinoma and metastatic adenocarcinoma. It is not useful in the differential diagnosis between HCC and benign hepatocellular mass

lesions. Although good general sensitivity has been reported, it is higher in well or moderately differentiated HCC that present less problems regarding the differential diagnosis with cholangiocellular carcinoma or metastasis [67]. Cytoplasmic stain is not observed in healthy liver or benign neoplasms; it is characteristic of malignancy but seen mostly in cholangiocellular carcinoma and metastatic neoplasms. The rate of cytokeratin fraction expression is 15% for CK7, 20% for CK20 and 10% for CK19. Diffuse strong expression of endothelial markers CD31 and CD34 is not characteristic for normal liver tissue in contrast to HCC [27]. The visualisation of endothelial layer is valuable also in estimating the thickness of trabeculae. However, pattern of diffuse, strong endothelial marker expression has low sensitivity of 20-40%. The patchy expression is also difficult to evaluate in liver biopsies. The visualisation of endothelium thus is not recommended for the distinction between adenoma and carcinoma [6].

The transcription factor TTF-1 is expressed as intense granular cytoplasmic staining in normal liver parenchyma [16] and hepatocellular tumours (Figure 3). The reaction is ensured by cross-reactivity with hepatocyte mitochondrial antigen and is seen with the clone 8G7G3/1 [69]. The reported sensitivity is 60-70%. However, it parallels the expression of Hep Par 1 decreasing the practical value [6]. Its expression can be retained even in metastatic HCC [16].

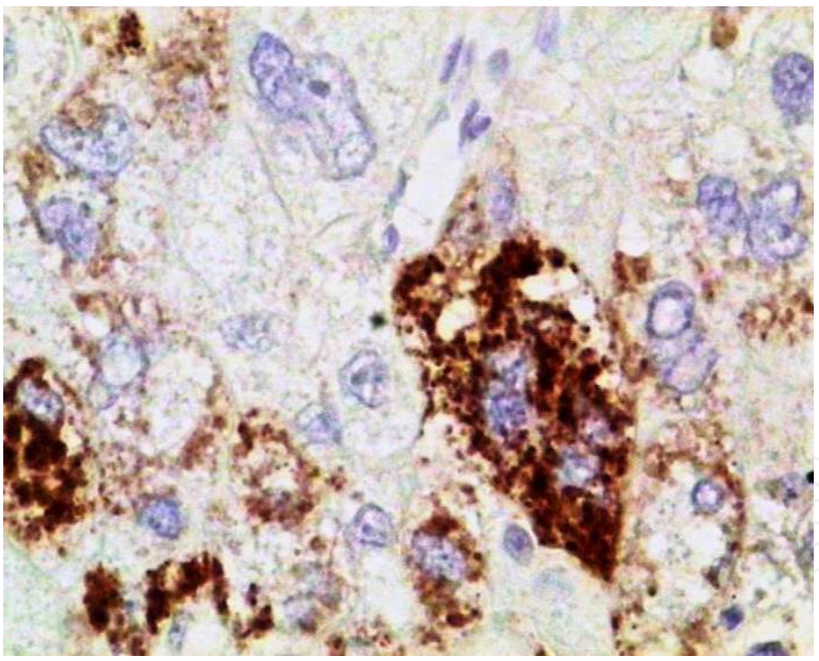


Figure 3. Granular cytoplasmic expression of TTF-1 in hepatocellular carcinoma. IP, Anti-TTF-1, OM 400x.

MOC-31 is an epithelial cell surface glycoprotein of unknown function. Evaluating liver biopsies, it is valuable as non-hepatocellular marker. MOC-31 is negative in HCC but positive in most metastatic adenocarcinomas and cholangiocellular cancer [67]. However, mesothelioma is MOC-31 negative as well; calretinin should be used in the panel to exclude this possibility [17].

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Molecular subtyping is emerging for HCC. The subtypes are distinguished by high proliferation and chromosomal instability; by activation of Wnt signalling pathway and by interferon signalling due to tumour-infiltrating cells [70-77].

The requests for clinically relevant classification have resulted in the separation of HCC into early and progressed entities. The early HCC is recognized as small (not exceeding the diameter of 2 cm), well differentiated and lacking vascular invasion. The invasion into portal tracts can be present and is highlighted by lack of proliferating ductules. Macrovesicular steatosis is present in 40% of early HCC but appears mostly in Eastern cohorts. It can be attributable to incomplete neoarterialisation – the process of portal tract replacement by unpaired arteries outside the portal tracts. In early HCC, there is still comparatively large venous flow. The tumours in general may be radiologically hypovascular. The early HCC is more likely to become the biopsy target due to equivocal findings at imaging. Progressed HCC includes HCC of higher grade (moderate or poor differentiation degree, G2 or G3), possessing vascular invasion, larger size or stem/progenitor cell immunophenotype and mixed hepatobiliary differentiation. The stem cell immunophenotype can be detected by IHC for CK19, EpCAM, CD133, and mixed hepatobiliary immunophenotype – by expression of CK7 and CK19 [7]. The 5-year survival is 89% in the early HCC group in contrast to 48% in the progressed group. The intrahepatic metastatic spread must be distinguished from multifocal carcinoma that is prognostically better disease. The multifocal disease is characterised by "nodule in nodule" structure or by presence of at least one G1 nodule [7].

The differential diagnosis includes benign hepatic lesions, metastatic malignancies and cholangiocarcinoma. IHC is of major importance. Markers, that are expressed both in benign and malignant liver cells (CEA by polyclonal antibody, CD10, Hep Par 1, TTF-1 and (occasionally) cytokeratins [27]) identify the hepatocellular origin of tumour but cannot be used to prove the malignant biological potential of suspicious biopsied tissue. If these are found in high-grade tumour, diagnosis of HCC is preferable in contrast to metastasis. The expression of alpha-fetoprotein and glypican-3 is typical for malignant tumour of hepatocellular origin [27]. These findings are important in differential diagnosis with non-hepatocellular and/or metastatic tumour in line with other markers specific for particular histogenesis. Regarding the differential diagnosis of HCC and dysplastic cirrhotic nodule, a panel of immunohistochemical stains is recommended employing glypican-3, glutamine synthetase and heat-shock protein 70 [48,78-80]. In biopsy, the panel has lower sensitivity although good specifity: accuracy 60.8% for 3 markers and 78.4% for 2 markers with 100% specifity. The findings were acceptable even in the group of low-grade HCC: the accuracy still was 57% for 3 markers and 72.9% for 2 markers with 100% specifity [23].

HCC (except fibrolamellar type) mostly is associated either with cirrhosis or chronic active hepatitis with fibrosis that has not reached the degree of cirrhosis. To facilitate the differential diagnosis between HCC and liver adenoma or FNH it is wise to take separate biopsies-from the lesion and from distant liver tissues if possible.

The future pathways for molecular diagnostics of HCC include mRNA analysis of *GPC3*, *survivin* and *LYVE1* genes [78]. Glypican-3, encoded by *GPC3*, and survivin is up-regulated

in parenchymal HCC cells while LYVE1 protein is down regulated in endothelial cells in case of malignancy. MYC pathway studies could also bring new information [29].

In addition, molecular studies can predict the HCC prognosis. Down-regulation of p57 accelerates the growth and invasion of HCC cells [18]. The reduced p57 expression correlates with larger tumour size, higher TNM stage, presence of extrahepatic metastases and decreased survival. In cell lines, the down-regulation of p57 increases the expression of cyclin D1 and CDK2, enhancing the cellular proliferation. The matrix metalloproteinase-1 (MMP-1) and protease activated receptor-1 (PAR-1) are expressed in HCC but not in normal liver. The up-regulation of MMP-1/PAR-1 axis has prognostic value [20] and potentially could be used in the identification of malignancy. Co-expression of stem cell transcription factors Oct4 and Nanog indicates aggressive tumour behaviour and predicts recurrence after HCC resection [22]. FOXJ1 is over-expressed in HCC. It is associated with histological grade, poor prognosis and with tumour cell proliferation [19]. Hedgehog signalling pathway mediates invasion and metastasis of HCC via ERK pathway. Up-regulation of cell proliferation is associated with down-regulation of p27 and p21 and up-regulation of cyclin D1 [81]. Osteopontin plays role in the proliferation of HCC through interaction with the cell surface receptor CD44 [82] and is considered the key mediator for vasculogenic mimicry [83]. Bax-interacting factor is over-expressed in HCC and correlates with shortened survival [84]. NY-ESO-1 protein is a potential marker for early recurrence after surgical treatment [85]. Hepatocyte nuclear factor 4 suppresses the HCC development [86]. Sulfatase 2 protects HCC cells against apoptosis [87]. Interleukins as IL-17 and IL-6 have tumour-promoting role [88]. Interaction with matrix metalloproteinases 2 and 9 is likely [89]. Up-regulation of sirtuins has been identified [90]. Typing of immune cells in biopsy is mostly done for research purposes [91]. If any of those parameters will show prognostic and predictive value, the relevant IHC analysis should be included in the protocol of liver biopsy evaluation. The technological future developments include virtual microscopy. Fractal analysis [92] and quantitative IHC can be applied [93].

Methylation studies have been carried out in HCC [94]. The expression of microRNAs is undergoing active analysis in HCC [95-96]. MicroRNAs are non-coding, short RNA molecules that can bind to messenger RNA and to prevent their translation into protein, providing additional regulation of gene expression. MicroRNAs act as large-scale molecular switch due to ability simultaneously down-regulate many genes. MicroRNA-181 down-regulates the differentiation and maturation of hepatocytes [96]. Suppression of microRNA-181 expression leads to reduced motility and invasion of HCC stem cells [25]. MicroRNA-182 could promote metastasis [97]. MicroRNA-183 inhibits apoptosis [98]. MicroRNA expression can be subjected to regulation with IL-6 [25]. Reduced expression of microRNA-26 in HCC is associated with poor prognosis. However, better response of interferon alpha postoperative adjuvant therapy can be expected [95]. MicroRNA-21 induces resistance to the anti-tumour effect of interferon and fluorouracil combination therapy [99]. Circulating microRNAs are valuable in tumour diagnosis and monitoring the treatment [24].

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3.2. Hepatoblastoma

Hepatoblastoma is defined as a primary malignant blastomatous liver tumour showing complex differentiation towards fetal and embryonal hepatocytes as well as mature tissues including osteoid, connective tissue and striated muscle. Epidemiologically, hepatoblastoma is a rare malignant liver tumour of childhood with the incidence of 1 case / 1 million [8,10]. In children, hepatoblastoma is the most common primary liver tumour. Characteristically, the tumour develops within first five years of life: 4% of hepatoblastomas are present at birth, 69% have developed by 2 years of age and 90% - by 5 years of age. Only 3% of patients are older than 15 years [100]. The risk of hepatoblastoma is increased in *APC*-mutation-carrying children from familial adenomatous polyposis (FAP) kindreds. Clinically, enlarging abdomen can be the first sign. The other possible manifestations include weight loss, anorexia, nausea, vomiting and abdominal pain. Jaundice is rarely observed [100]. Paraneoplastic syndromes can occur. Among those, anaemia and thrombocytosis are frequent. Precocious puberty due to production of chorionic gonadotropin is rare. Grossly, the tumours mostly occur as single lesions [10] measuring 5-22 cm [100]. Pseudocapsule can develop due to compression of surrounding liver tissue. Microscopically, hepatoblastoma can display any of different histological patterns, or combination of these patterns. The fetal epithelial differentiation is characterised by thin trabeculae of small cuboidal cells. The nuclei are small and round with fine chromatin and small nucleolus. The cytoplasm can be either clear or finely granular resulting in "light and dark" pattern under low magnification. Foci of extramedullary haemopoesis can be present. The combined fetal and embryonal pattern is characterised by presence of small tumour cells in solid or acinar groups. The small cells have scant cytoplasm, higher nucleo: cytoplasmic ratio and coarse chromatin. Hepatoblastoma is called macrotrabecular if the cells compose 6-12 cell layers in the trabeculae in most of the tumour. Larger cells are present in the macrotrabeculae in addition to fetal and embryonal type. In teenagers, macrotrabecular hepatoblastoma must be differentiated from hepatocellular carcinoma. Small cell undifferentiated hepatoblastoma morphologically resembles small cell cancer displaying solid small blue cell pattern with focal necrosis. Mixed epithelial and mesenchymal hepatoblastomas contain mesenchymal components including fibrous tissue, osteoid, cartilage, striated muscle, bone or melanin [100]. Mixed epithelial and mesenchymal hepatoblastoma with teratoid features is recognised by the presence of endodermal, neuroectodermal and complex mesenchymal tissues. The neuroectodermal component can comprise melanin, glial and neuronal cells [10].After treatment, connective tissue, necrosis and signs of haemorrhage develop in association with residual neoplastic tissue, and squamous islands become more common. Immunohistochemically, expression of alpha-fetoprotein, beta-catenin and cell cycle markers is associated with the histological pattern. The fetal subtype is characterised by low proliferation that parallels the scant mitotic activity; alpha-fetoprotein can be present and the expression of beta-catenin is retained in the membranous localisation. The combined fetal and embryonal subtype is characterised by shift of beta-catenin expression towards the nuclei in higher grade embryonal component. An interesting circular pattern can be observed. In the rounded cell groups, the middle is occupied by progenitor-type pale, small cells displaying low proliferative activity and nuclear expression of beta-catenin. The progenitor-type cells are surrounded by intensively proliferating embry-

onal type cells characterised by mixed nuclear and cytoplasmic expression of beta-catenin. The outermost layer of these concentric structures is composed by fetal type cells with low proliferative activity and retained membranous expression of beta-catenin. The small cell subtype lacks alpha-fetoprotein but has high proliferative activity, usually reaching 80%; cytokeratins are expressed as well. Even in the mixed epithelial and mesenchymal hepatoblastoma, cytokeratins and alpha-fetoprotein can be expressed even in the ostecyte-like and osteoblast-like cells embedded in or associated with the osteoid, correspondingly [10]. In the study of Purcell *et al*., cyclin D1 and Ki-67 were two markers (out of 5, including also beta-catenin, E-cadherin and alpha-fetoprotein) that were shown to have prognostic value regarding survival [8].

3.3. Cholangiocarcinoma

Cholangiocarcinoma (CC) is defined as malignant epithelial liver tumour with biliary histogenesis or biliary differentiation. Epidemiologically, CC is a rare tumour with male predilection. It composes 15% of primary liver cancer [100] but the relative incidence range of cholangiocarcinoma is wide, from 5% in males and 12% in females in Osaka, Japan, to 90% in males and 94% of primary liver cancer cases in females in Thailand. The age-standardized incidence per 100 000 males ranges from 84.6 in Thailand to 2.8 in Osaka, Japan; 1.0 in France or 0.9 in Italy. The known risk factors include association with ulcerative colitis and primary sclerosing cholangitis [27]. The rate of cholangiocarcinoma in primary sclerosing cholangitis patients is estimated as 10-20%. The presence of parasites, especially *Clonorchis sinensis* and *Opisthorchis viverrini*, also increases the risk of cholangiocarcinoma. The high-incidence area in Laos and North and Northeast Thailand corresponds to the endemic area of *Opisthorchis viverrini*. Korea has high rate of cholangiocellular cancer due to endemic spread of *Clonorchis sinensis.* Clinically, the patients can present with painless jaundice [31], general malaise, mild abdominal pain and weight loss [100]. Grossly, several types exist. Peripheral tumours arise from portal bile ducts. Hilar lesions arise in large ducts. The diffuse intraductal papillomatosis involves ducts as widespread carcinoma *in situ* lacking dominant mass but leading to severe obstruction of bile flow. Histologically, cholangiocarcinoma has adenocarcinomatous structure characterised by tubular complexes and moderate amount of desmoplastic stroma. The architectural variants include high-grade tumour lacking the characteristic architecture, signet-ring cell tumour with presence of signet-ring cells, mucinous type with extensive secretion of extracellular mucin, adenosquamous type with focal squamous differentiation and spindle cell type with pseudosarcomatoid structure, presence of malignant spindle cells and signs of epithelial differentiation. The tumour has no functional connection with bile excretory system although morphological connection in the form of invasion or cancer in situ can exist. CC arises from ductal epithelium and not from hepatocytes. Due to these two reasons, presence of bile in the lumina of malignant glands is not characteristic but eosinophilic or mucinous secretion can be present. Mucin stains as PAS or mucicarmine can be positive [44]. The immunophenotype is derived from the immunophenotype of bile duct epithelium, with expression of following cytokeratins: CK19 (100%), CK7 (80-100%), CK20 (20%). Diffuse cytoplasmic expression of CEA is found by polyclonal antibody in almost all cases and is frequent by monoclonal antibody as well [27]. However, it is

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suggested that morphology cannot reliably distinguish cholangiocarcinoma from metastatic pancreatic or colorectal cancer [31]. In case of pancreatic adenocarcinoma, the marked cellular atypia disproportionally to better preserved architecture can be a clue. Colorectal adenocarcinoma in typical cases is characterised by columnar morphology and diffuse intense expression of CK20, CDX2 and CEA and lack of CK7. Other authors have drawn attention to the impossibility to distinguish cholangiocarcinoma from metastatic gastric cancer and cancer of gall bladder; metastatic pancreatic cancer also remains a problem [6]. The morphological differential diagnosis includes benign proliferation of bile ducts, hepatocellular carcinoma and metastatic adenocarcinoma [27]. In order to discriminate between biliary adenoma and cholangiocarcinoma, invasion (including single invasive cells and perineural invasion) and cellular atypia should be sought for. Radiologic findings are helpful as bile duct adenoma usually is smaller than 1 cm, but cholangiocarcinomas are large. The differential diagnosis with hepatocellular carcinoma can rely both on morphology and immunophenotype. Immunohistochemically, markers of biliary differentiation CK7 and CK19 are positive in cholangiocellular carcinoma. Hep Par 1 can be used to exclude hepatocellular differentiation [6,29]. Proteomic analysis of differentially expressed proteins in peripheral cholangiocarcinoma is under research [101].

4. Vascular tumours

Cavernous haemangioma, epithelioid haemangiendothelioma and angiosarcoma are endothelial tumours representing the whole spectrum of biological potential. Haemangioma is entirely benign although can cause complications due to large size; epithelioid haemangioendothelioma is notable for the peculiar structure leading to marked difficulties in the biopsy diagnostics, and angiosarcoma is a frank malignancy with grave prognosis. In addition, angiomyolipoma will be discussed as well although it should be noted that this tumour has complex structure including rich vascularity as one component.

4.1. Cavernous haemangioma

Haemangioma is defined as benign endothelial tumour [102]. Due to bleeding risk, it is only rarely seen in liver biopsy; in addition, the possibilities of radiological diagnostics are good and the prognosis only rarely necessitates active treatment. However, epidemiologically the lesion is the most common benign tumour of the liver with incidence 0.4% [27]. Clinically, haemangioma usually are asymptomatic due to small size and slow expansive growth. Occasionally, a giant haemangioma (10-30 cm) can cause pain due to mass effect. Thrombosis and bleeding can be dangerous complications. In neonates, blood shunting can lead to heart failure. Grossly, haemangiomas are mostly solitary (90%), of small or moderate size (less than 5 cm) and subcapsular. Microscopic structure is similar to cavernous haemangioma elsewhere in the body. Cavernous, lake-like blood spaces can be seen, separated by hypocellular fibrous septa (Figure 4). Thrombosis can be present. The immunophenotype reflects

the endothelial origin. In the rare situation, when biopsy is obtained from cavernous haemangioma, the differential diagnosis can include hepatic tumours with rich vascularity as adenoma and cholangiocellular carcinoma. These are diagnosed by the presence and cytological properties of liver cells. Other vascular tumours could be considered, including infantile haemangioendothelioma, angiomyolipoma, epithelioid haemangioendothelioma and angiosarcoma. The infantile haemangioendothelioma can be recognized by capillary structure and occurrence in infants [27]. Angiomyolipoma shows combination of fat, smooth muscle and blood vessels with radiating immature smooth muscle cells. The higher cellularity and presence of fat are features incompatible with cavernous haemangioma. Epithelioid haemangioendothelioma is discussed separately; the occurrence of vascular lakes usually is not observed. Angiosarcoma can have cavernous architecture but the hallmark of it is the cellular atypia.

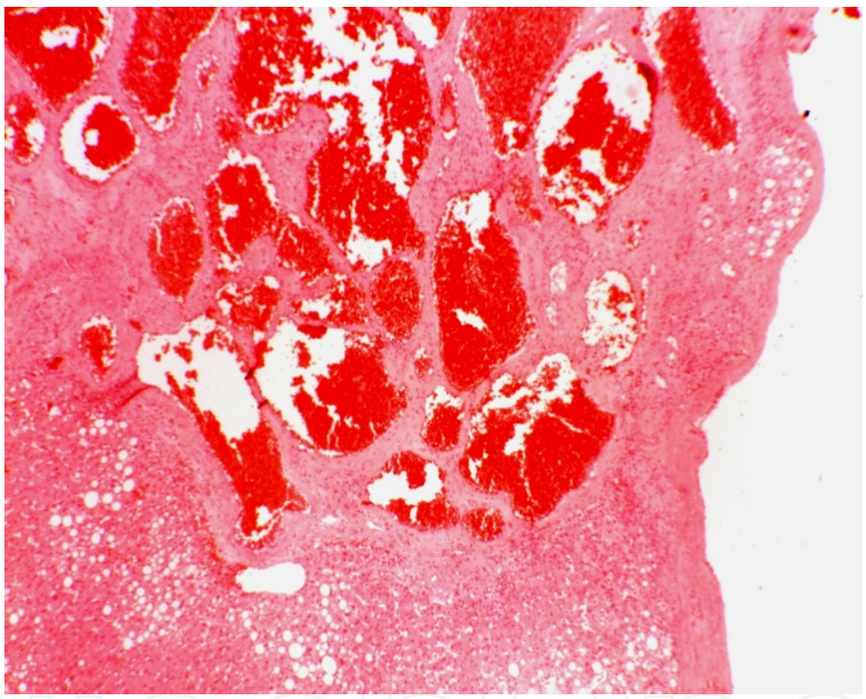


Figure 4. Cavernous haemangioma in liver tissue. Note the large, cavernous spaces filled with red blood cells. HE, OM 50x.

4.2. Angiomyolipoma

Angiomyolipoma is defined as benign mesenchymal tumour with complex structure including immature smooth muscle, blood vessels and fat. Epithelioid cells and perivascular HMB-45-positive cells can be present. Research of the tumour histogenesis has resulted in the concept of PEComa, a tumour of perivascular epithelioid cells, showing myomatous, lipomatous and melaninogenic differentiation. Epidemiologically, liver angiomyolipoma is rare. It has been diagnosed in wide age range (10-86 years). In tuberous sclerosis, the inci-

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dence of angiomyolipoma is increased. These patients may develop multiple angiomyolipomas in liver as well as kidney angiomyolipoma. Awareness of this condition is necessary to escape over-diagnosis of metastatic malignant tumour. Clinically, the tumour can be asymptomatic. However, large tumours can cause pain; rupture and bleeding is also possible. By radiologic studies, the tumour is hypervascular again. Grossly, angiomyolipoma usually is solitary (except in tuberous sclerosis), measuring 0.8-36 cm. The microscopic picture (Figure 5) is straightforward if all three components are present in liver biopsy and have typical structure. The smooth muscle cells can have epithelioid appearance leading to morphological similarity to liver parenchymal cells; the rich vascularity could lead to diagnostic confusion with hepatocellular tumour already earlier. The epithelioid cells sometimes can cause suspicion for malignancy due to large nuclei and nucleoli. However, the nucleo: cytoplasmic ratio remains low due to increased cell size.

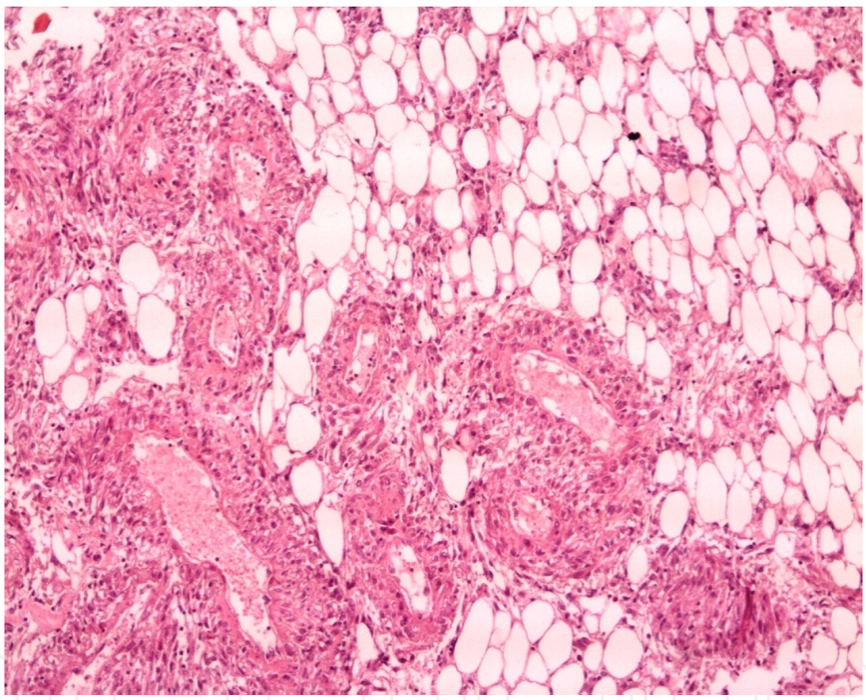


Figure 5. The microscopic structure of angiomyolipoma. Note the peculiar, thick-walled blood vessels, immature smooth muscle proliferation with high cellularity as well as the presence of fat. HE, OM 100x.

In difficult cases, IHC is helpful. The smooth muscle cells express actin (Figure 6) and fat cells – S-100 protein. HMB-45 expression can be observed in perivascular epithelioid cells (Figure 7). The differential diagnosis can include hepatocellular neoplasms or spindle cell sarcomas. Actin expression and complex histological structure helps to exclude hepatocellular origin of the tumour. Complex structure, combined immunophenotype and low proliferation help to exclude sarcoma [27].

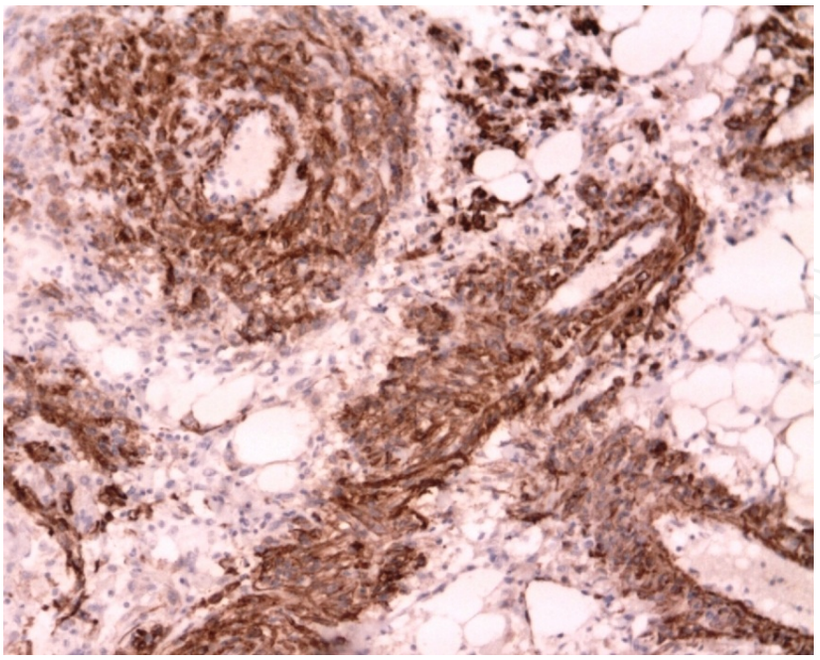


Figure 6. Actin-positive smooth muscle component in angiomyolipoma. IP, anti-actin, OM 100x.

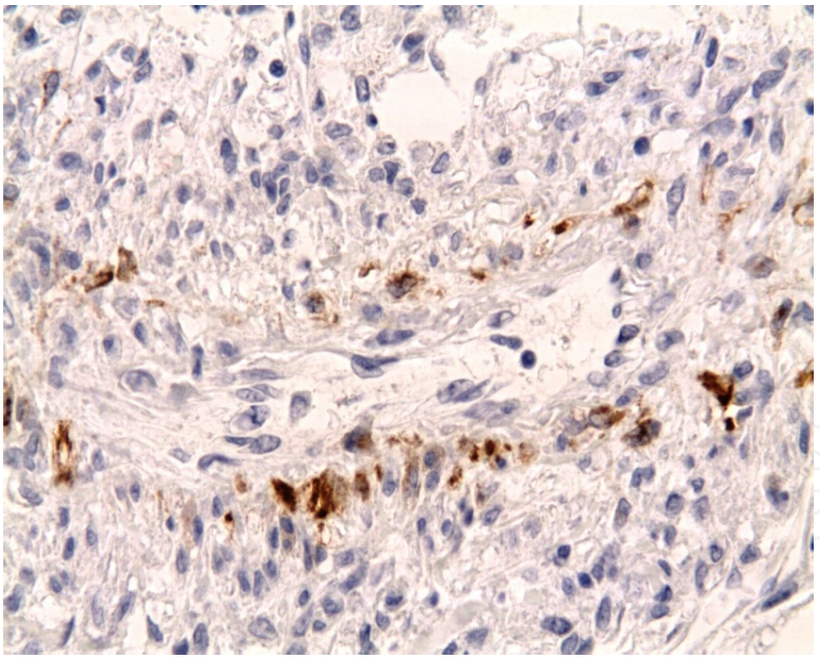


Figure 7. Expression of melanosome protein HMB-45 in angiomyolipoma. IP, anti-HMB-45, OM 400x.

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4.3. Epithelioid haemangioendothelioma

Epithelioid haemangioendothelioma (EHE) is defined as intermediate-grade malignancy derived from endothelial cells. The mean age of patients is 47 years, ranging 12-86 years. The clinical picture can include symptoms related to enlarging mass in liver (abdominal pain, hepatomegaly) and tumour-related intoxication (fatigue, malaise, anorexia). Radiologically, the tumour can be found by computed tomography. EHE can be radiologically avascular [10,27,103]. This finding is probably related to fibrosis and scarcity of functioning blood vessels despite the endothelial origin of the tumour. Grossly, multiple tumours can involve liver or liver and lungs. In the lungs, epithelioid haemangioendothelioma is known also as intravascular bronchioloalveolar tumour. Despite the multifocality (Figure 8), slow progress is possible in our experience.

Biopsy material is usually sufficient to diagnose the tumour. However, in our experience, immunohistochemical investigation is crucial in order to find out the presence of tumour cells on the background of stromal fibrosis and reactive inflammation, to detect the endothelial origin and to evaluate the low biological potential as reflected by low to moderate proliferation activity by Ki-67 (Figures 9-11).

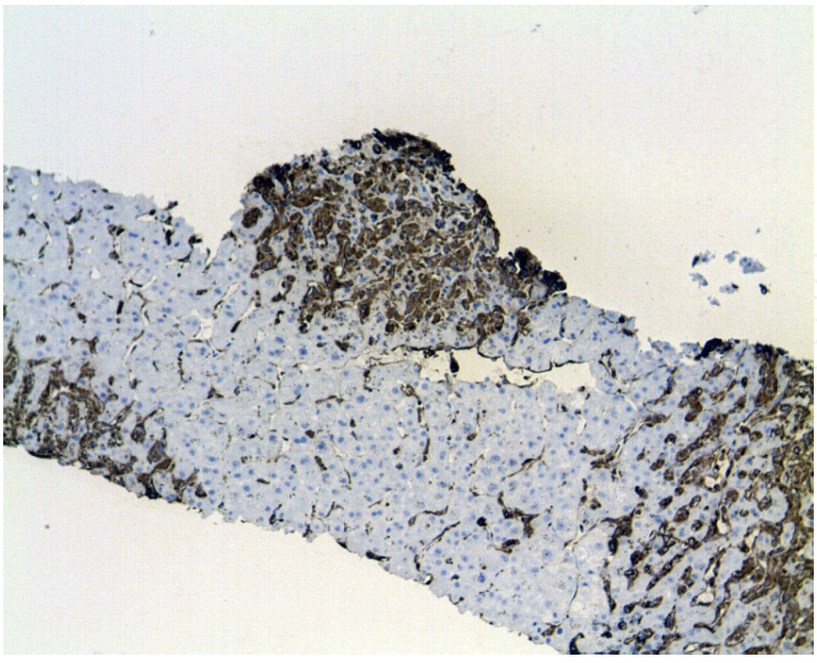


Figure 8. Multiple foci of epithelioid haemangioendothelioma in liver biopsy. The tumour is highlighted by immunohistochemical visualisation of vimentin regarding its mesenchymal nature. IP, anti-vimentin, OM 50x.

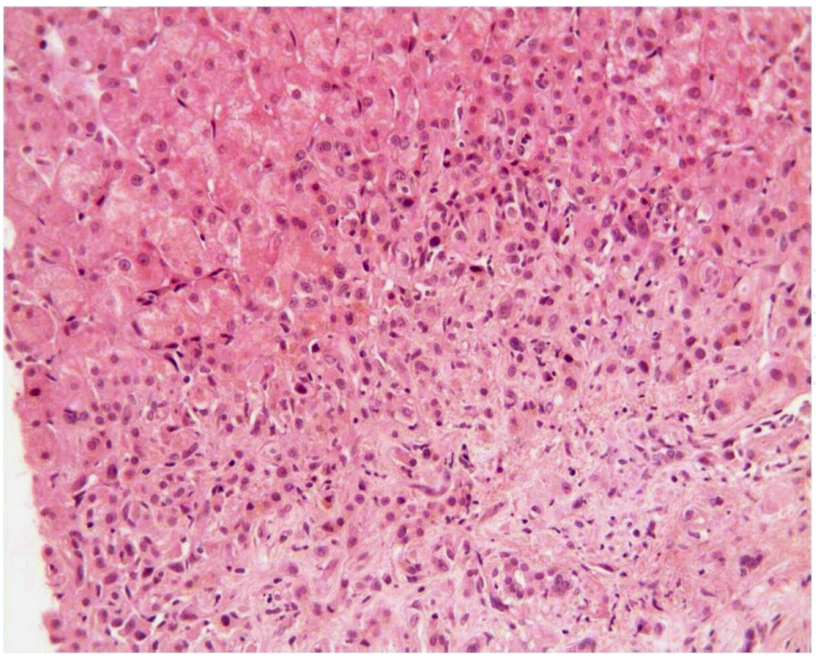


Figure 9. Epithelioid haemangioendothelioma presenting as a fibrotic focus in liver biopsy. HE, OM 100x.

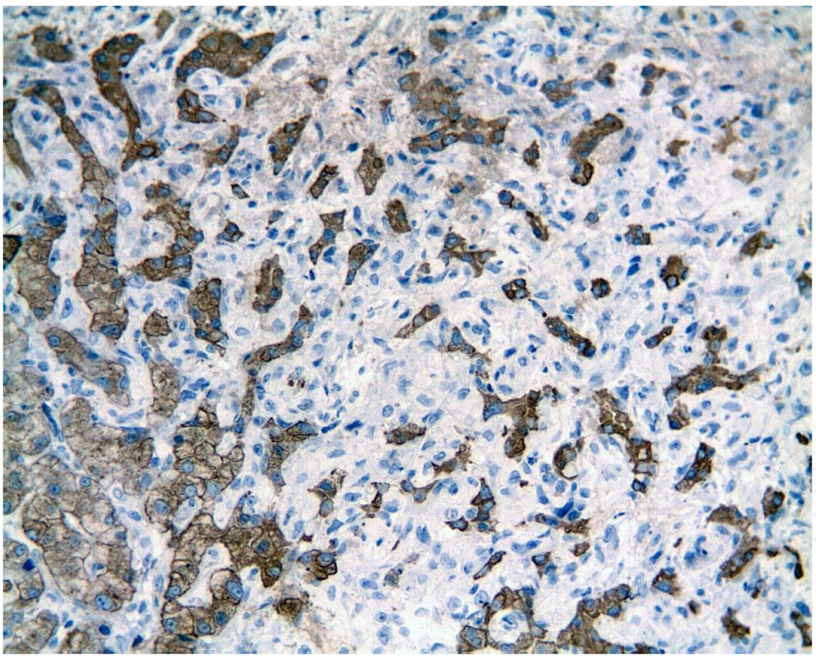


Figure 10. Loss of liver parenchyma due to infiltration of epithelioid haemangioendothelioma. IP, anti-cytokeratins AE1/AE3, OM 200x.

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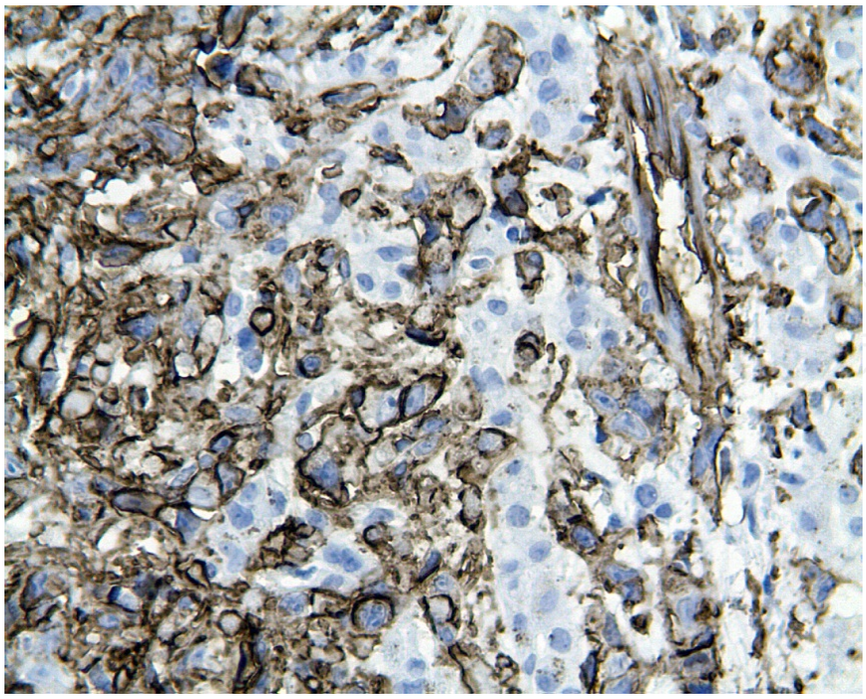


Figure 11. Expression of CD34 in epithelioid haemangioendothelioma. Note also the positive reaction in the lining of a venule. IP,anti-CD34, OM 400x.

The tumour is growing within sinusoids and venules compressing the adjacent parenchyma. As was mentioned, the expression of endothelial markers is typical. Focal expression of cytokeratin and/or actin is possible [103] and should not cause confusion if panel of immunostains is performed. Stromal fibrosis follows than and can become marked so that neoplastic cells are obscured (Figure 9). Two cell types are described: epithelioid and dendritic. The morphological differential diagnosis includes non-neoplastic fibrosis and/or inflammation and granulation tissue, angiosarcoma and metastatic cancers with marked stromal fibrosis. The non-neoplastic conditions can be ruled out by tumour architecture as revealed by immunohistochemistry. Epithelial tumours can be excluded by the predominance of endothelial markers by IHC. Among the vascular malignancies, the diagnosis of epithelioid haemangioendothelioma is preferred for lesions with low grade atypia, absence of frankly malignant spindle cells, low proliferation, limited destruction of surrounding liver tissue and absence of necrosis.

4.4. Angiosarcoma

Angiosarcoma is defined as malignant tumour of endothelial cells. Epidemiologically, it is characterised by rare occurrence in the liver constituting 2% of primary hepatic malignancies [11]. Elderly (50-60-year-old) males represent the largest group of affected patients [27]. The described risk factors include history of thorotrast use for arteriography, exposure to vinyl chloride in the plastics industry where it has been used for polymerisation, arsenic compounds (used as insecticides, possibly present in wine and used in the treatment of psoriasis), copper compounds, pesticides and other chemical carcinogens. In all cases, long

latent period (6-35 years) embarrass the data collection. The clinical picture can show signs and symptoms of liver damage (hepatomegaly, local pain, jaundice), disorders of blood cell function (anaemia, thrombocytopenia, disseminated intravascular coagulation), and tumour-related intoxication manifesting as weight loss. Ascites, bleeding into abdominal cavity and liver failure is possible [27]. Grossly, multiple masses with signs of haemorrhage are present. Morphologically, the cellular atypia as well as vascular differentiation can be observed in variable extent. High-grade tumours exhibit solid growth with few vascular spaces. Immunohistochemically, endothelial markers CD31 and CD34 are expressed. However, the immunophenotype can be not straightforward. In our experience, it is important to use several endothelial markers. At first, the reactivity can be uneven [27]. Even more, CD34 is technologically beneficial antibody characterised with high affinity. However, during the evaluation it is necessary to consider CD34 expression in non-endothelial tumours including gastrointestinal stromal tumour and solitary fibrous tumour, among others.

5. Metastatic liver tumours

In Western countries, metastatic tumours represent the most common malignant liver lesion with the rate 94-98% among all malignant liver tumours [27]. Almost all malignant tumours, including carcinomas, sarcomas, melanomas and haematological malignancies, can secondary involve the liver by haematogenous, lymphogeneous or transperitoneal spread. Theoretically, metastatic tumour retains the morphological characteristics of the primary site. However, the balance between anaplasia and differentiation can shift towards anaplasia in such degree that signs of differentiation towards specific tissue or cell type are hardly recognisable. Some tumours like squamous cell cancer and melanoma lack specifity regarding the organ of origin. Even adenocarcinomas retain few specific features. Therefore the differential diagnostics between primary and secondary liver tumours represents a complicated practical task. Clinical data can be absent if metastatic liver lesion presents as cancer of unknown origin. The diagnosis can be reached by logical analysis of morphology, IHC and molecular data. If the establishment of exact histogenesis is unsuccessful, the biopsy investigation should be directed towards the analysis of treatment possibilities. Pathologist should comment in detail morphological and immunophenotype data that could either prove or disregard any particular type of treatment.

In case of liver metastasis, the primary tumour most frequently is located in colon, pancreas, stomach, breast, oesophagus, genitourinary organs [100, 103]. Lung cancer can metastasize to liver as well [6]. Neuroendocrine tumours, even small, can give rise to hepatic metastases. The clinical course in this case can be prolonged and occasionally characterised by carcinoid syndrome including flushing, diarrhoea and palpitations.

The spectrum of metastatic tumours in liver biopsies depend on the frequency of different tumours, the biological properties of different neoplasms predicting the possibility of metastatic spread to liver as well as by the medical paradigm considering the indications

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for liver biopsies. In the files of single university hospital, metastatic tumours constituted 45% of tumours or tumour-like liver lesions. Adenocarcinoma was the most frequent histological type of metastases (65.5%) comprising metastases of colorectal (48.2%), pancreatic (13.5%), breast (13%), gastric (6.2%), lung (4.5%) and oesophageal cancer (3.7%). Neuroendocrine carcinomas were seen frequently (16%). Lymphoma constituted 0.4% of all tumours [2]. Metastases in cirrhotic liver were rare [2]. In another study, including 130 cases of metastatic liver disease, gastrointestinal tract was found to be the most common primary location (45.3%) of cancer metastasizing to liver followed by neuroendocrine tumours (10.7%) [104]. In children, neuroblastoma, nephroblastoma and rhabdomyosarcoma are the most frequent source of metastases [103].

The spread to liver occurs in 5-10% of patients with Hodgkin's lymphoma and 15-40% of non-Hodgkin's lymphoma cases at the time of diagnosis. Leukemias can involve the liver as well. Grossly, large cell lymphoma can form masses similarly to carcinoma. In case of Hodgkin's disease, the size of nodules is variable. Leukemic infiltrate can be present without visible mass lesion. Myeloid leukemias preferentially infiltrate sinusoids, lymphoid – portal tracts, but hairy cell leukemia can involve both portal tracts and sinusoids forming small blood containing cavities, surrounded by neoplastic cells [103].

Malignant melanoma (Figures 12-14) is one of the greatest challenges in diagnostic surgical pathology [105] due to amelanotic, clear cell, sarcomatoid, small cell, haemangiopericytoid, signet-ring cell, myxoid, metaplastic and rhabdoid forms. The diagnosis largely depends on IHC. Evaluating the intermediate filaments, melanoma expresses vimentin. Despite the reported concerns of cytokeratin expression in melanoma, this is rare event (3%) in formalin-fixed tissues. Similarly, the expression of glial fibrillar acidic protein and actin is observed in 1% of melanomas [105]. Interspersed normal cells should be excluded from evaluation of cytokeratin and actin reactivity. Melanoma is characterised by nuclear and cytoplasmic expression of S-100 protein in 97.4-98%. S-100 protein can be observed in carcinomas, histiocytic neoplasms and malignant peripheral nerve sheath tumour, therefore melanoma-specific antibodies, e.g., HMB-45 and MART-1/Melan-A must be included in the panel. Melanoma can express bcl-2, CD10, CD68, CD56, CD57, CD99, CD117 antigens leading to diagnostic confusion with lymphoma, renal cell cancer, hepatocellular cancer, GIST, seminoma and other neoplasms. Expression of Melan-A is found also in metastatic adrenocortical carcinoma (50-60%) that can be recognised by inhibin expression in around 70% of cases [6]. S-100, HMB-45, Melan-A and inhibin are absent from HCC [6].

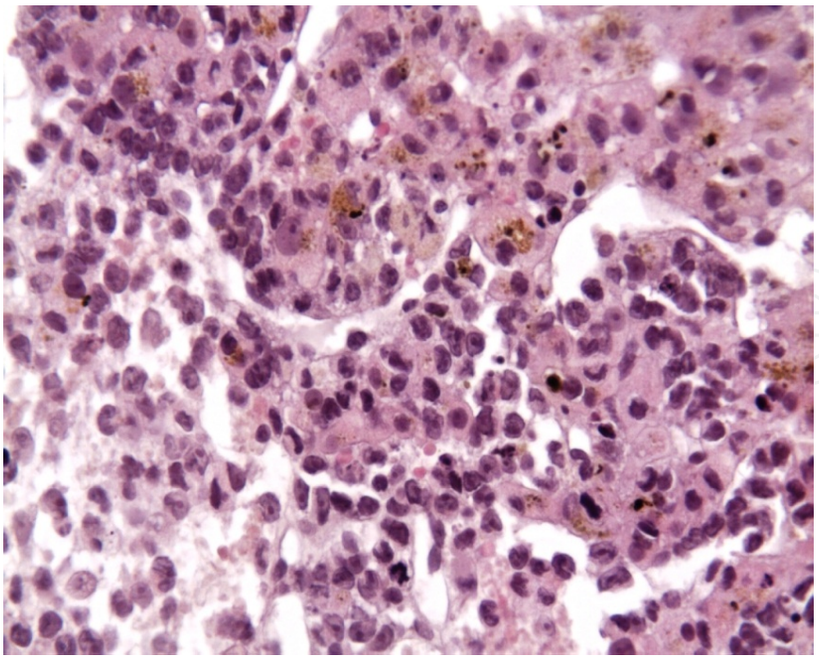


Figure 12. Diffuse sinusoidal spread of undifferentiated malignant tumour. By immunohistochemistry, metastatic melanoma was revealed (see also Figure 13). HE, OM 400 x.

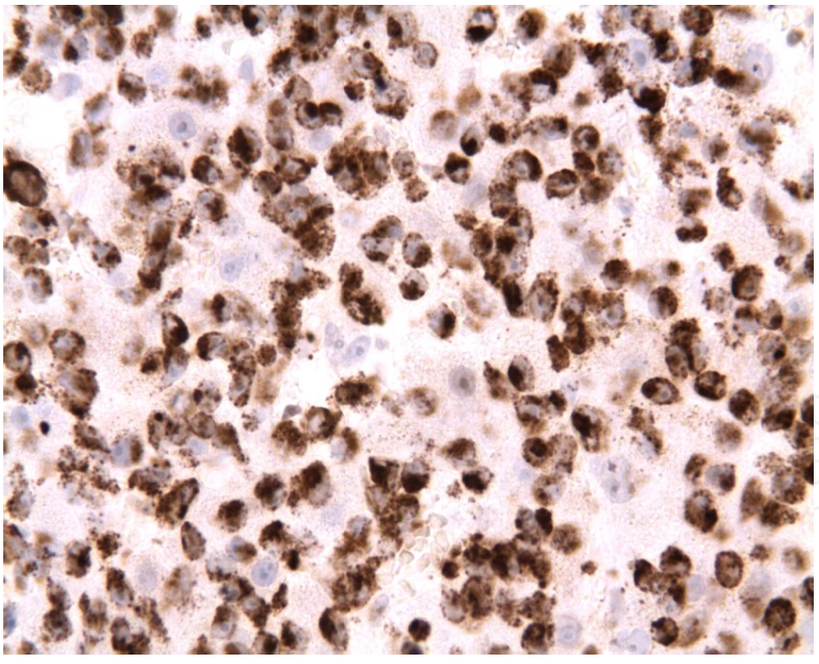


Figure 13. Intense perinuclear expression of melanosome protein HMB-45 in metastatic melanoma. IP, anti-HMB-45, OM 400x.

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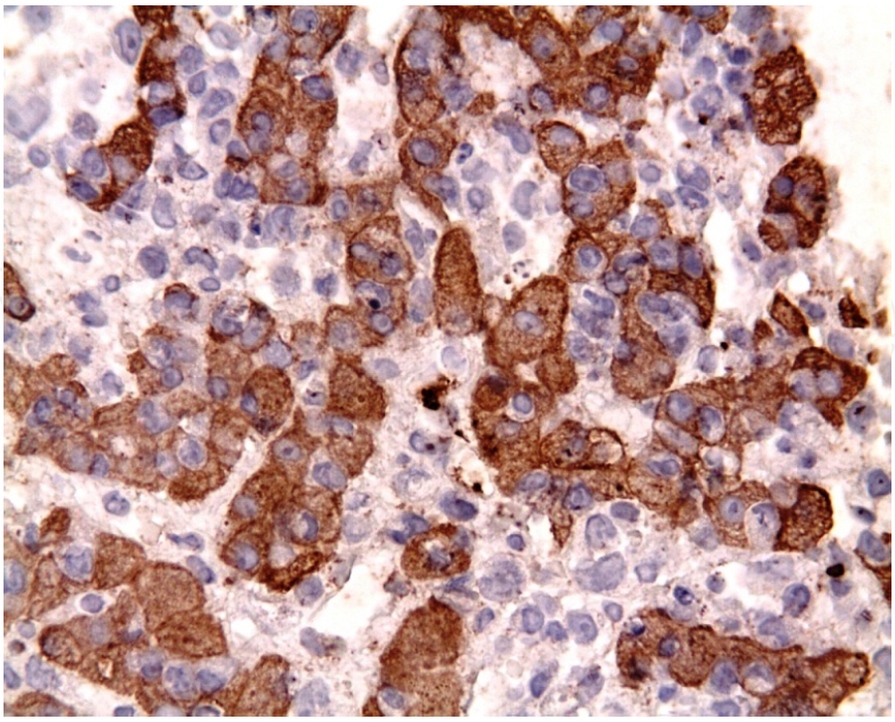


Figure 14. Lack of cytokeratins AE1/AE3 in metastatic melanoma. Note the unusual sinusoidal spread. IP, anti-AE1/AE3, OM 400x.

Metastatic breast cancer expresses CK7 but not CK20. However, this immunophenotype is shared by many adenocarcinomas. To identify the tumour as metastasis from breast primary tumour, gross cystic disease fluid protein fraction-15 (GCDFP-15) and/or mammaglobin can be detected. The specifity of GCDFP-15 is estimated as 99%, and the sensitivity ranges from 50 to 74%. Breast cancers of luminal molecular type express oestrogen (ER) and progesterone receptors (PR). Naturally, the expression of female steroid hormone receptors is shared by ovarian and endometrial cancer. Nowadays the detection of ER and PR is routine in breast cancer diagnostics but less experience is obtained with expression of hormone receptors in extra-genital carcinomas. The scientific studies report expression of ER in carcinoma of lung, stomach and thyroid [105]. The cross-reactivity can be associated by certain antibody clones. Also, HER-2 positive and triple negative molecular types of breast cancer are more prone to develop visceral metastases. Thus, negative ER/PR expression cannot exclude metastatic breast cancer, and positive findings should be interpreted with caution recognising the possibility of metastatic ovarian or endometrial cancer and cross-reactivity or true expression of hormone receptors in extra-genital tumour. ER/PR expression in lung or thyroid tumour can be controlled by TTF-1 protein expression and/or evaluation for neuroendocrine markers and calcitonin.

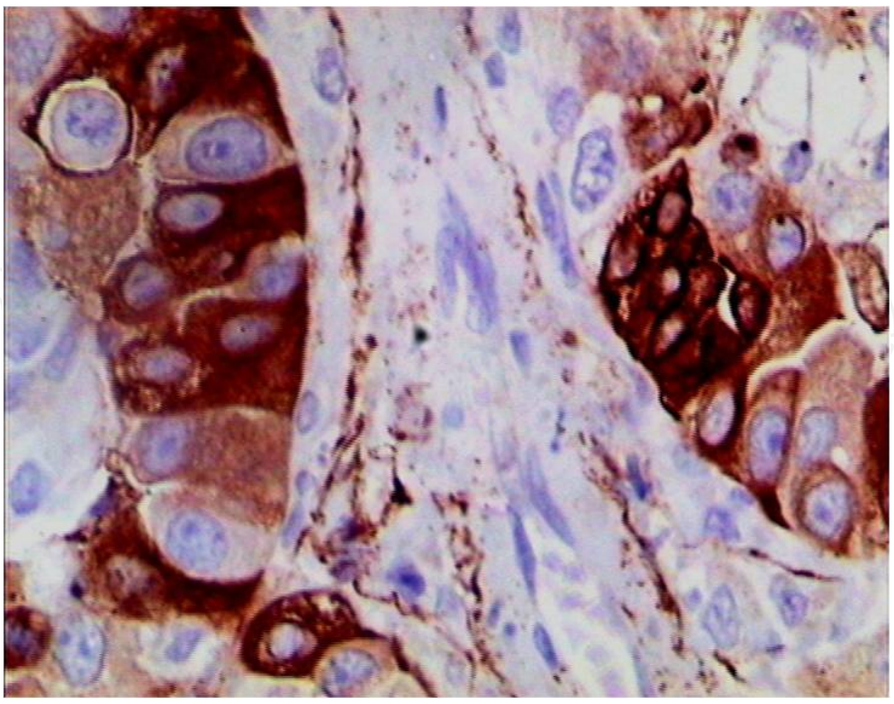


Figure 15. Surfactant A in pulmonary adenocarcinoma. IP, anti-surfactant apoprotein A, OM 400x.

Adenocarcinoma, squamous cell cancer, small cell cancer and carcinoid are the most frequent lung neoplasms. Lung adenocarcinoma is characterised by expression of CK7 (100%) and TTF-1 (60-75%). Expression of CK20 is rare. Cytokeratins 5/6 and 34betaE12 can be present but are not dominant in comparison with CK7. Vimentin can be found in lung adenocarcinomas. Nuclear expression of TTF-1 and/or cytoplasmic expression of surfactant apoprotein A (Figure 15) is an evidence of pulmonary origin. Small cell cancer expresses neuroendocrine markers and pan-cytokeratin. The expression of chromogranin A and CK AE1/AE3 can be limited to perinuclear dot reactivity. Simultaneous detection of leukocyte common antigen can be suggested to perform differential diagnosis with haematological neoplasm. Nuclear expression of TTF-1 protein is frequently present (Figures 16-18). The high proliferation fraction by Ki-67 is characteristic albeit unspecific. The immunophenotype of squamous cell cancer is unspecific and characterised by cytoplasmic expression of CK5/6 and CK 34betaE12 in association with strong nuclear reactivity with p63 protein. CK7 can be present but is not dominant. TTF-1 protein is absent. Carcinoid is characterised by neuroendocrine differentiation and low proliferative activity. The TTF-1 expression is not frequent [17,105-107].

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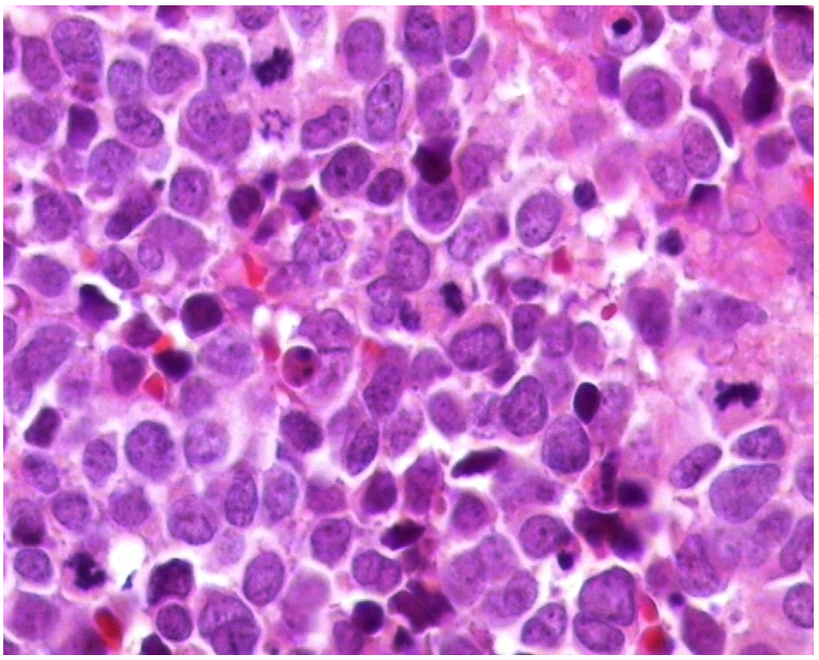


Figure 16. Small cell cancer. Note the "salt-and-pepper" chromatin and high mitotic activity. HE, OM 400x.

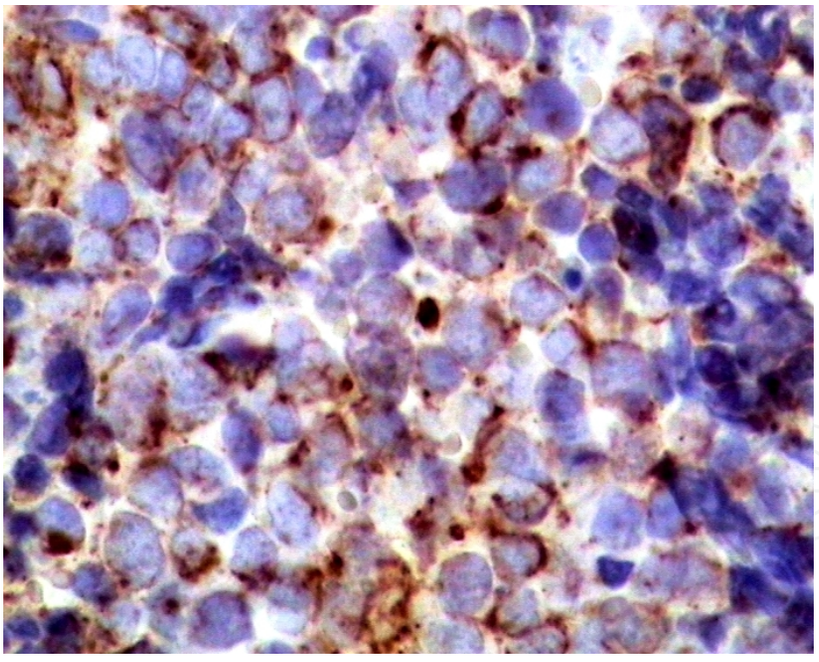


Figure 17. Granular cytoplasmic and perinuclear expression of chromogranin A in small cell cancer. IP, anti-chromogranin A, OM 400x.

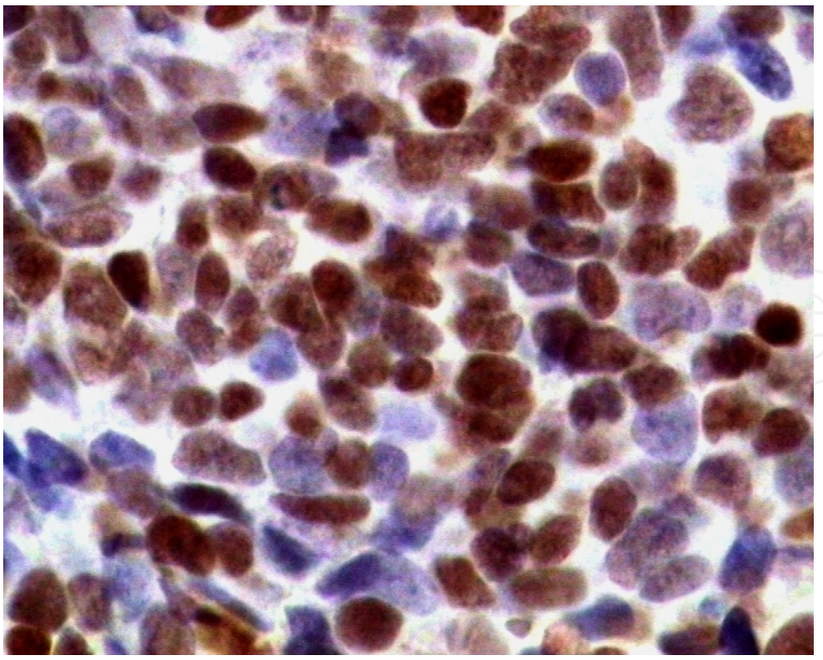


Figure 18. Nuclear TTF-1 expression in small cell cancer. IP, anti-chromogranin A, OM 400x.

Mesothelioma is characterised by expression of CK7, CK5/6, vimentin and calretinin (Figure 19). HBME-1 can be expressed as well but lacks specifity.

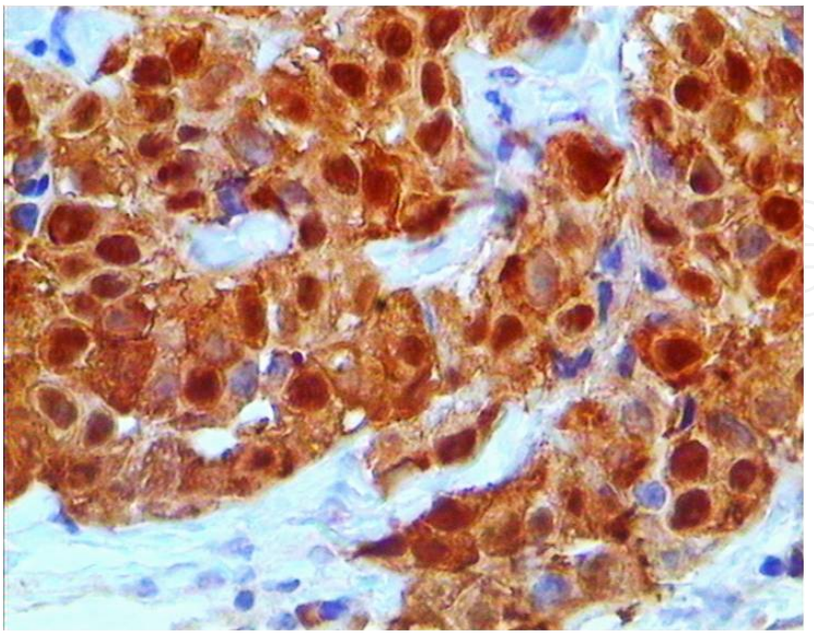


Figure 19. Nuclear and cytoplasmic expression of calretinin in epithelioid mesothelioma.IP, anti-calretinin, OM 400x.

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Metastatic colorectal carcinoma can be recognised by diffuse intensive cytoplasmic expression of CK20 and nuclear expression of CDX2 [108]. Carcinoid of the midgut and hindgut also are positive for CDX2 [109].

Neuroendocrine tumours are characterised by strong cytoplasmic expression of chromogranin A and synaptophysin and negativity for Hep Par 1 [6]. CD56 is considered to be the most sensitive neuroendocrine marker. In our experience, it shows reliable performance in small or compressed biopsies making it especially valuable tool for the evaluation of scant tissue material. Occasional CD56 expression in HCC is described [6].

Renal cell carcinoma is characterised by negativity for Hep Par 1 and CEA expression (by polyclonal anti-CEA antibody). Unfortunately, the rate of RCC expression decreases from 50-80% in primary clear cell renal carcinoma and 60-90% in papillary renal cell cancer to 20% in metastatic renal cell carcinoma. CD10 can be present both in HCC and renal cell carcinoma. Although the pattern of expression is different this can be difficult to evaluate, especially in core biopsy. PAX-2 is advised as marker of metastatic renal cell carcinoma with the expression rate 70-80%. Expression of vimentin is more characteristic in clear cell renal carcinoma (60-70%) than in hepatocellular carcinoma; chromophobe renal cell carcinoma also is negative [6].

As tumour heterogeneity remains a source of problems [69] and the immunophenotype can be inherently complex and subjected to cross reactivity, we recommend wide IHC panels including several markers for HCC and cholangiocarcinoma as well as markers for metastatic tumour, including the organospecific antigens (see Tables 1-2).

Tumour	Immunophenotype
Malignant melanoma	Vim + CK AE1 / AE3 – S-100 + HMB-45 + MART-1 / Melan A +
Lung adenocarcinoma	CK7+ CK20- CK34betaE12-/+ TTF-1+ Surfactant apoprotein A +
Small cell cancer	CK AE1/AE3 + ChrA+ CD56 +TTF-1 +
Squamous cancer	CK34betaE12+ CK7-/+ CK20 – p63+
NET	CK AE1/AE3 + ChrA+ TTF-1 +/ - (lung) or CDX2 + (midgut, hindgut)
Breast cancer	CK 7 + CK20 – MG +/– ER +/– PR +/–
Colorectal cancer	CK20+ CK7- CDX2+ TTF-1-

Table 1. The immunophenotype of selected malignant tumours. Abbreviations in the Table: Vim, vimentin; CK, cytokeratin; TTF-1, thyroid transcription factor 1; ChrA, chromogranin A; NET, neuroendocrine tumour; MG, mammaglobin; ER, oestrogen receptor; PR, progesterone receptor

Antigen	Valuable positive expression	Notes
Glypican-3	Hepatocellular carcinoma	Occasional positivity in non-hepatocellular tumour
Arginase-1	Hepatocellular carcinoma	Sensitivity for hepatocellular carcinoma 96% Normal liver tissue positive Metastatic tumours rarely positive [69]

Hep Par 1	Hepatocellular carcinoma	Sensitivity for hepatocellular carcinoma around 50% [69] Gastric carcinoma can be positive
AFP	Hepatocellular carcinoma	Sensitivity for hepatocellular carcinoma around 15% [69]
CD10	Renal cell carcinoma Hepatocellular carcinoma	Negative in adrenal carcinoma
CK7	Cholangiocellular carcinoma Metastatic cancers	Positivity does not exclude hepatocellular carcinoma Valuable for primary evaluation of malignant tumour within liver
CK17	Cholangiocellular carcinoma Metastatic cancers	Positive tumours as pancreatic cancer (58%), squamous carcinoma (75%), urothelial carcinoma (75%) and cholangiocellular carcinoma (35%) can be distinguished from negative ones (gastric, colorectal, prostate, breast cancer, hepatocellular carcinoma)
CK19	Cholangiocellular carcinoma Metastatic cancers	Positivity does not exclude hepatocellular carcinoma
CK20	Metastatic colorectal cancer	Useful in conjuction with CK7 for initial grouping of cancers showing adenocarcinomatous structure
CDX2	Metastatic colorectal cancer and NETs	Heterogeneous focal expression in gastric and pancreatic carcinomas Mucinous ovarian cancers can be positive Morules in endometrioid carcinoma are positive
Calretinin	Mesothelioma Adrenal cortical carcinoma Sex cord-stromal tumours of the genital tract	Squamous carcinoma frequently positive
Surfactant apoprotein A	Lung adenocarcinoma. In our experience possess high affinity and is useful, if positive	Reactivity in thyroid cancer (43% in small group) has been reported Heterogeneous expression has been observed [69]
TTF-1, nuclear expression	Metastatic pulmonary adenocarcinoma (75% of non-mucinous type and 10% of mucinous type), small cell cancer (pulmonary, 50-90%; non-pulmonary, 44-80%) or thyroid cancer including papillary, follicular and medullary but not anaplastic carcinoma [69]	Regarding pulmonary adenocarcinoma, less subjected to heterogeneity-related evaluation problems than surfactant apoprotein A Endometrial (17%) or breast (2.4%) cancer occasionally positive [69]
TTF-1, cytoplasmic expression:	Hepatocellular carcinoma	Expression in benign liver parenchyma is present Gastric or prostatic cancer can show cytoplasmic positivity
Chromogranin and synaptophysin	ANET	

http://dx.doi.org/10.5772/52838

CD56	NET, cholangiocarcinoma	Other tumours can be positive
Oestrogen and progesterone receptors	Breast, ovarian or endometrial cancer, endometrial stromal sarcoma	Non-gynaecologic cancers can be occasionally positive, including lung cancer (4-15-67%)
CD117	GIST Seminoma	CD34 is co-expressed in GISTs PLAP is co-expressed in germ cell tumours
Mammaglobin	Breast cancer	High heterogeneity Sensitivity for breast cancer 40-85% Ovarian (17%), endometrial (40-70%) and endocervical (30%) carcinoma can also be positive [69]
GCDFP-15	Breast cancer	Sensitivity for breast cancer 50-60% High heterogeneity Not associated with mammaglobin thus simultaneous evaluation can be recommended [69]
PSA	Prostatic cancer	Negative in 5% high-grade prostate cancers. Reactivity in few breast carcinomas and rectal NETs have been observed [69]
Pax8	Thyroid cancer Female genital tract carcinomas Renal cell cancer	Sensitivity for thyroid cancer 79-100% and for renal cancer 71-98% Breast cancer is negative. Positivity is useful to discriminate breast cancer from ovarian or endometrial cancer NETs can be positive
P63	Squamous cell cancer Urothelial carcinoma	

Table 2. Panel of antibodies for liver biopsy evaluation. Abbreviations in the Table: AFP, alpha-fetoprotein; CK, cytokeratin; NET, neuroendocrine tumour; TTF-1, thyroid transcription factor 1; GIST, gastrointestinal stromal tumour; PLAP, placental alkaline phosphatase; GCDFP-15, gross cystic disease fluid protein-15; PSA, prostate specific antigen

6. Cystic biliary tumours

The cystic biliary tumours are defined by cystic structure and development of / differentiation towards intrahepatic bile duct epithelium. The group includes malignant biliary cystadenocarcinoma and benign biliary cystadenoma. Epidemiologically, cystic biliary tumours represent rare entities, with incidence of biliary cystadenocarcinoma approximately 1/10 million (corresponding to 0.01/100 000) and of biliary cystadenoma 1/100 000 - 5/100 000 [110]. Biliary cystadenocarcinoma is diagnosed mostly at the age 50-60 years [100]. Biliary cystadenoma is diagnosed in younger patients: mean age 40.6 (range 30-51) vs. 51.3 (range 41-63) years in biliary cystadenocarcinoma group [14]. In other studies even larger age difference (17 years) is found between patients affected by benign and malignant cystic biliary tumours, respectively [111-112]. Cystic biliary tumours are more common in women: 80-100% of biliary adenoma and 63-71.4% of biliary cystadenocarcinoma are described in fe-

male [14]. The clinical picture reflects the presence of mass lesion and is dominated by abdominal pain [113]. The other manifestations and complications include jaundice, cholangitis, tumour rupture [114], haemorrhage [115], compression of the portal or caval veins with possible subsequent ascites [113], hemobilia [12] and mucobilia [116]. Notably, the tumour can progress slowly [117] with the clinical history of biliary cystadenocarcinoma as long as 10-15 years [112,118]. The long course is is in accordance with the low grade of malignancy and gradual development of tumour through stages of increased epithelial proliferation, dysplasia, *in situ* cancer and, finally, invasive cancer. Thus, long anamnesis of cystic hepatic mass does not exclude the possibility of malignant tumour and the need for careful follow-up if the cyst is not removed by operation. Although biopsy can be considered in cases with unclear differential diagnosis, it is not the first choice because of the following considerations. First, simple liver cyst is the main differential diagnosis of cystic biliary tumours. Although biliary cystadenocarcinoma is rare, liver cysts have high prevalence being present in 2.5% of the population [119] and cannot be distinguished from cystic biliary tumours on the basis of CA19-9 and CEA levels [14,114]. However, core biopsy is unlikely to yield sufficient tissue in case of simple cyst or cystadenoma; it also is not suitable for the diagnostics of focal malignancy and rarely can lead to peritoneal carcinomatosis [13]. Therefore radiological diagnostics, especially computed tomography, is essential [117]. Grossly, biliary cystadenocarcinoma is multicystic. Internal mural nodules are irregularly distributed in the walls. The tumour most frequently is located within the liver (83%). Extrahepatic bile ducts (13%) or the gall bladder (0.02%) has been affected by this tumour as well [14]. The size of cystic biliary tumours (1.5-30 cm) is not helpful in the differential diagnostics between simple hepatic cyst and cystic biliary tumours; it also has no correlation with malignant biological potential [120]. The metastatic spread of biliary cystadenocarcinoma can affect the liver, regional lymph nodes in the hepatoduodenal ligament, lungs, pleura or peritoneum [100]. Histologically, biliary cystadenocarcinoma is characterised by clear-cut signs of malignancy: cellular atypia, particularly nuclear polymorphism, mitotic activity and invasion into surrounding stroma. The tumour architecture is cystic and papillary. The benign counterpart of biliary cystadenocarcinoma, the biliary cystadenoma lacks the malignant features [100] and is composed by either mucinous or serous benign epithelium. Most of cystic biliary tumours possess characteristic mesenchymal, ovarian–type stroma. Hypothetically, these tumours arise from bile ducts proximal to the hilum of the liver and share the cystic structure and presence of peculiar ovarian-type mesenchymal stroma with mucinous cystic tumours of the pancreas and retroperitoneum, leading to the hypothesis that ectopic ovarian stroma during embryogenesis can become incorporated along the biliary tree, in the pancreas and retroperitoneal space and cause the proliferation of the adjacent epithelium by production of the hormones and growth factors [121]. Origin from intrahepatic peribiliary glands [122] or from ectopic rests of primitive foregut sequestered in the liver [114] has been hypothesised. Development from pluripotential stem cells is suggested on the basis of the presence of albumin messenger RNA and biliary type cytokeratins in the tumour cells [123]. Biliary cystadenocarcinoma without mesenchymal stroma more frequently arises in males and carries poorer prognosis in comparison with the tumour possessing mesenchymal stroma [122]. By immunohistochemistry, increasing proliferative activity by Ki-67 ex-

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pression as well as increasing p53 protein expression from adenoma to carcinoma was shown in biliary cystadenocarcinoma without ovarian-type stroma [124]. Expression of cytokeratin (CK) 7 and absence of CK20, CEA, alpha-fetoprotein, calretinin, CD31 and chromogranin is described [125]. However, presence of CK20, although typical for colorectal cancer, is described in cholangiocarcinoma, especially non-peripheral [126]. It might be expected in biliary cystadenocarcinoma with growing awareness about this entity.

There is evidence showing that at least some cases of biliary cystadenocarcinoma originate from pre-existing biliary cystadenoma. These data include the age difference between biliary cystadenocarcinoma and biliary adenoma patients [14] as well as morphologic findings of malignant transformation in a lesion with focally innocuous structure [127].

Radiologically, presence of internal septations allows excluding a simple cyst. Vascularity of septa is characteristic for cystic biliary tumours [14] and is considered by some specialists to be more reliable in distinguishing biliary cystadenoma from cyst than the simple presence of septations [117]. Biliary cystadenoma is characterised by smooth and thin internal septa, but presence of enhanced mural nodules in the outer wall or septa is the most important sign of malignancy. Calcification is not frequent but has been found specific for malignancy by some [14] but not all [119] authors as far as cystic biliary tumours are concerned. Size, number of septations or location of the neoplasm does not help to differentiate between benign or malignant cystic biliary tumours [14]. Some authors have postulated that preoperative differentiation between biliary adenoma and cystadenocarcinoma by radiologic imaging is not possible therefore liver resection should be performed for all cystic biliary tumours [120]. This assumption is based on the experience that internal papillae with arterial enhancement may be present in both tumours so that computed tomography and magnetic resonance imaging yield overlapping data.

The clinical differential diagnosis of cystic liver lesions, entering the differential diagnosis of biliary cystadenocarcinoma, include developmental, neoplastic, inflammatory and traumatic lesions as simple bile duct cyst, polycystic liver disease, biliary hamartoma, cystically degenerated cases of other primary or metastatic liver tumours, abscesses, hydatid cyst, extrapancreatic pseudocyst, hematoma and biloma [119,128].

7. Conclusions

In conclusion, wide variety of neoplastic processes can affect the liver. Most of non-cystic tumours can be reliably diagnosed in liver biopsy. Several demographic and clinical data should be submitted along with the liver biopsy. Patient's age and presence or absence of clinical symptoms must be known. If there is history of contraceptive use it should be reported. Radiological data have high relevance: the size, localisation in respect to liver capsule and number of focal liver lesions should be known to the pathologist. The vascularity should be described. Knowing these data, pathologist should evaluate the haematoxylin-eosin stained specimen. Wide panel of immunohistochemical stains can be recommended than.

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